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Serologic and skin-testing data on a group of patients having cat scratch disease are presented to demonstrate a possible relationship to the psitt-LGV group of viruses. In addition, the results of skin-testing patients with different batches of skin-test antigen are given.

The data obtained indicate that the incidence of positive serologic reactions with the psitt-LGV group antigen is consistently higher in patients with cat scratch disease than in individuals of the control group. However, the percentage of positive reactions is not what would be expected from any direct etiologic causal relationship.

The response of groups of individuals to different preparations of skin-testing antigen was so variable as to suggest that either more than one agent may be involved or marked strain variations must occur among the agents producing this clinical syndrome. In a small series of LGV patients, 2 of 5 did not respond with positive skin reactions when tested with cat scratch antigen, and at least 2 of the remaining 3 responded in a manner difficult to interpret.

It is now apparent that the clinical syndrome referred to as cat scratch disease (CSD) or fever (nonbacterial lymphadenitis, benign inoculation lymphoreticulosis, etc.) is of considerable importance. Since its recognition in 1932 by Foshay, an increasing number of cases have been reported. Recent description of several large patient series emphasizes the fact that the disease is more prevalent than was originally considered (1-4). Furthermore, it is now conceded that cat scratch disease, because of its extreme protean nature, is often not recognized as a specific entity but is frequently confused with other clinical syndromes—especially those producing a lymphadenopathy.

Most investigators are of the opinion that this disease is of viral etiology. Confirmation of this consideration remains obscure, however. Mollaret and his associates (5) reported the successful transmission of the disease to humans and monkeys by inoculation of lymph node material from cases of CSD. Zwissler (cited by Warwick and Good (6)) indicated that the disease could be reproduced in humans with material from suppurative nodes. The ability to produce positive skin reactions in cat scratch patients with material passaged in rat testes and chick yolk sacs was also indicated. More recently the presence of a "viral hemagglutinin" was described and isolation of an agent suggested (7). Few of the investigators have been successful in their attempts to isolate the virus.

It has been advocated that the inciting agent of CSD belongs to the psittacosis-lymphogranuloma venereum (psitt-LGV) group of viruses. Thus, Mollaret et al. described the production of inclusion bodies identical with those described for psittacosis. Kalter and associates (3) were, however, unable to confirm the occurrence of intracytoplasmic inclusions. Daeschner et al. (1) referred to the

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feline pneumonitis virus as a possible factor in causation of CSD.

Relationship with the psitt-LGV group of viruses has also been postulated on the basis of the complement-fixation (CF) test, by use of a group antigen. Apparently there is disagreement in results, or more accurately in interpretation of results, when employing the CF test for diagnostic assistance. Mollaret and his group, using lygranum® as the test antigen, found that 46.5 percent of their cat scratch patients responded with titers of 1:10 or higher in the CF test. Armstrong et al. (8) questioned the significance of these titers, although their own data showed a greater percentage of positives among patients having cat scratch disease than was found in control groups.

In a study of CSD in New Zealand, Manning and Reid (9), using a lygranum® antigen, substantiated the correlation between positive cat scratch skin tests and positive skin reactions. In a series of 35 patients with positive skin reactions, these investigators found 23 percent positive response (titers of 1:8 or higher). There were no positive CF reactions among 44 patients reacting negatively to the cat scratch skin test, and only 2 percent of 50 control serums, obtained from normal blood donors, showed any evidence of antibody. Spaulding and Hennessy (4) similarly found that about 40 percent (14 of 39) of their patients responded with positive CF reactions. A control group of 120 persons failed to demonstrate any antibody to the test antigen. Morrissey (10) found 7 of 10 positive skin reactors and 2 of 8 persons with negative skin-test reactions to have titers of 1:32 or greater to this group antigen.

Agreement is more uniform regarding the use of the skin test in diagnosis and its relationship to this illness. The skin test seemingly is the most specific test available, although infrequent false reactions are encountered. Unfortunately, the skin test is not without its shortcomings. Individuals remain positive for many years, probably for life. This introduces a potential error, although it is advocated that the skin test be used to help establish diagnosis only when there is also a clearly defined clinical picture. In addition, it is now evident that patients vary greatly in their reaction to different preparations of skin-testing antigen.

This study was initiated to ascertain the value of the CF test as a laboratory aid in making a diagnosis of CSD. An attempt was made also to evaluate the diagnostic significance of skin-testing antigens by the simultaneous testing of a single patient with different preparations of antigen.

MATERIAL AND METHODS

In order to obtain sufficient material for serologic evaluation of the CF test and mass skin testing, a program supplying physicians with skin-testing antigen was instituted. In return, the following information and materials were requested:

1. Age of patient.
2. Extent of adenopathy.
3. Incubation period (i.e., time of appearance of adenopathy in relation to association with cats).
4. Occurrence of disease in any other member of the family.
5. Acute and convalescent blood samples.
6. Results of skin test.
7. Suppurative material, if available.

As a result of this program, antigen was prepared in quantity sufficient for testing several thousand patients. In many instances the requests were ignored, with subsequent loss of desired information and material.

The data reported here were accumulated from 130 acute and convalescent serums representing this number of patients and 40 single convalescent serums. A control group of 200 serums from untested individuals, selected to give only comparable age groups, was included. Approximately 75 percent of the study group were children; the remaining serums were derived chiefly from young adults, and a few from older people. Since age did not

appear to have any direct relationship to the results reported here, no attempt was made to separate the test groups.

Serology

All serums were inactivated at 56° C. for one-half hour and tested in the CF test routinely employed in this laboratory. A psitt-LGV group antigen was used at its optimal dilution as predetermined in a box titration. Concurrent titrations with control serum insured the maintenance of antigen titer. A final test volume of 0.6 ml. was obtained by employing 0.1 ml. quantities of each reagent except complement, which consisted of 2 units in 0.2 ml. A number of serums were retested in modifications of the CF test without alteration of results.

Skin-testing antigens

Approximately 30 different batches of antigen (prepared similarly as the Frei antigen) have been made and distributed. These antigens, after sterility tests in the usual bacteriologic media and mice (suckling and adult), were stored in rubber-stoppered vials at 4° C. Many of the preparations were still active after storage in this manner for at least three years.

Sterility tests for possible inclusion of hepatitis virus are not available. Screening of all donors for a history of hepatitis remains the only known protective device against this group of agents. The possible use of ultraviolet irradiation for sterilization has not been studied.

Before a new lot of skin-testing antigen was introduced, it was tested simultaneously with a known preparation. Variations in erythema-producing capabilities were frequently encountered. Two lots were discarded because they did not produce a recognizable area of erythema when tested along with antigens known to be positive.

Skin tests were performed by inoculating 0.1 ml. of antigen intradermally into the forearm. Readings were made at 24 and 48 hours, as variation in time of appearance of reaction has been repeatedly encountered. Any area of erythema, regardless of size, was considered positive. Induration alone, on the other hand, was considered of questionable significance. Immediate reactions, which did not persist, were considered negative.

RESULTS

Serologic

All serologic tests were performed without prior knowledge of the skin-test results. When quantities permitted, serums were tested in two different serology laboratories, where similar results were obtained.

Of the 130 pairs of acute and convalescent serums tested with the psitt-LGV group antigen, 6 showed a fourfold or higher rise in CF antibody (table I). There were 4 patients that demonstrated increases of doubtful significance in antibody titers. Twenty-four other patients manifested titers, 12 of which were at the level of 1:4. In contrast, 96 patients failed to exhibit detectable antibody to this antigen. The results obtained by testing the 40 single specimens were approximately the same as with the paired serums. Three percent (6 of 200) of the control serums indicated CF antibody to this group antigen, and these were all at either the 1:4 or the 1:8 level.

The titers observed in individuals exhibiting antibody responses are difficult to interpret. Of the 24 patients with antibodies but no rise in titer, only 2 had titers of 1:64 and these were both in the acute phase specimen (table II). Drop in titer between acute and convalescent phase specimens was shown more often than increased titers. It is evident that the antibody response, as determined by using a psitt-LGV group antigen, is of low order. Although not indicated, the titers of individuals developing a fourfold or higher antibody response were also rather low (i.e., ranging between 1:8 and 1:64).

TABLE I

Complement-fixation results on serums from patients with cat scratch disease (positive skin reactions)

	Number tested	Number with 4-fold rise	Number doubtful rise	Number with stationary antibody	Number without antibody
Paired serums	130	6 (4.6%)	4 (3.1%)	24 (18.5%)	96 (73.8%)
Single serums	40	X	0	10 (25%)	30 (75.0%)
Controls*	200	X	0	6 (3.0%)	194 (97.0%)

*Randomly selected serums from individuals not skin tested with cat scratch disease antigen.

TABLE II

Complement-fixation titers of serums demonstrating **no** *rise in titer*

	CF titer*					
	<4	4	8	16	64	Total
Paired serums						
Acute	2	12	0	8	2	24
Convalescent	8	12	0	4	0	24
Single serums	0	7	1	2	0	10
Controls	194	4	2	0	0	200

*Reciprocal of dilution.

Skin tests

The results of comparative skin-test studies, with different batches of antigen, indicated a wide variation in response (table III). Among 4 patients simultaneously tested with antigens 1 and 2, positive reactions were shown by 3 patients and 4 patients, respectively (group 1). A similar observation was made on 5 patients tested with preparations 2 and 3 (group 2). Three patients tested with antigens 1 and 3 (group 3) reacted as follows: one was positive with No. 1 and negative with No. 3; the second patient demonstrated the reverse of this (i.e., he was positive with No. 3 and negative with No. 1); and the third patient was positive with both antigens. Two patients (group 4) were tested with No. 6; one of these was tested also with No. 2, and the other, with No. 5. Both of these individuals were positive to No. 6 but negative to the other antigen.

Another test group (group 5) consisted of 4 volunteers who were simultaneously tested with four different lots of antigens. These tests were made in the usual manner, two inoculations were given on each arm but without any predetermined area for any particular antigen. All 4 patients reacted in a positive manner, although differences in sizes of reactions appeared to three of the antigens (Nos. 5, 6, and 8); however, 2 of the patients were negative to test antigen 3.

Results of test groups 6 and 8 show how extreme the variation can be in reaction to the different lots of antigens. One antigen, No. 7, was consistently negative on 10 of 12 patients. Positive reactions were observed only on the donor of the suppurative material and on another completely unrelated patient. Antigen 8 was positive in 10 cases but negative on the 2 patients reacting with antigen 7. Five patients tested with antigens 7, 8, and 10 reacted only to Nos. 8 and 10 (group 7). Equally indicative of the variation in response to different skin-test antigens were the findings with Nos. 10 and 11. After assuming that lot 10 was satisfactory (based upon results

TABLE III

Skin-test reactions to preparations of antigens from different patients

Test group*	Antigen preparation 1	2	3	5	6	7	8	10	11
1	3/4†	4/4							
2		4/5	5/5						
3	2/3		2/3						
4		0/1		0/1	2/2				
5			2/4	4/4	4/4		4/4		
6						2/12	10/12		
7						0/5	5/5	5/5	
8								6/25	23/25
Total	5/7	8/10	9/12	4/5	6/6	2/17	19/21	11/30	23/25

*Each test group was simultaneously tested with the designated antigen preparations. See text for full explanation.

†Numerator represents number of individuals positive; denominator represents number of individuals tested.

obtained with antigens used in test group 7), we tested 25 patients with antigens 10 and 11 simultaneously (group 8). Of the 25 patients in the group, 4 were positive with both No. 10 and No. 11; 23, including the 4 positive with No. 10, were positive with No. 11; and 2 patients were positive to No. 10 but negative to No. 11.

Interpretation of these results is difficult, and no reasonable conclusion can be drawn. It may be noted that all antigens were tested in at least two different groups. In most instances, good correlation between antigens from different patients was obtained; however, certain exceptions were noted. Although, as indicated above, any reaction, regardless of size of erythematous area, is considered positive, it is obvious that extreme variations in size of reaction may be encountered. In certain instances, patients responded with barely detectable wheals (measurable in millimeters), while others reacted to a more marked degree. Large erythematous reactions, measured in centimeters or even inches, were frequently noted. Not infrequently, a reaction similar to that observed at the site of the scratch (i.e., the development of a papule) was seen. These at times may leave a permanent scar at the site of the skin test. It is highly probable that, in most instances, individual differences in sensitivities account for these variations. The results obtained, however, may also be interpreted as antigenic differences among the preparations.

Skin tests of LGV patients

An attempt was made to define the relationship of cat scratch disease to the psitt-LGV group of agents by skin-testing known positive LGV patients. Accordingly, arrangements were made to test LGV patients with cat scratch and Frei antigens. In addition, CF tests with the psitt-LGV group antigen were performed on the serum from these individuals (table IV). All 5 patients, 4 of which were Frei-positive, reacted in the CF test with titers greater than 1:10, with a high of 1:160. The skin reactions with the cat scratch antigen were not conclusive; only 1 patient responded to cat scratch material with a clearly defined

TABLE IV

Cat scratch skin tests, Frei tests, and complement-fixation results on lymphogranuloma venereum patients

Patient No.	Cat scratch skin reaction	Frei test	CF test
1	+	+	1:160
2	±	−	1:160
3	−	+	1:80
4	+*	+	1:20
5	−	+	1:10

*Negative until the seventh day and then positive.

area of erythema. The results on 2 patients were negative and on 2 others, questionable. Very rarely are questionable reactions observed inasmuch as any area of erythema is considered positive. It is interesting to note that 1 patient with a questionable cat scratch skin test was negative with the Frei antigen but had significant demonstrable antibody to the CF group antigen. One may speculate, since definitive information is not available, that the Frei test was negative as seen in early cases or perhaps reversed as observed following cortisone therapy. The other questionable patient may have been a case of delayed sensitivity in response to the skin-test material. This reaction would be similar to those reported by McGovern et al. (11) in which induration and erythema were not observed until 5 to 9 days after administration of the skin test.

DISCUSSION

In favor of a relationship between cat scratch disease and the psitt-LGV group of agents is the continued relatively high incidence of patients demonstrating antibodies to the group antigen; the consensus among laboratories reporting CF results indicates a greater frequency of group antibodies among cat scratch patients than among control groups (table V). In certain instances the numbers involved are too small to be significant. It is apparent, however, that there is generally a greater frequency of reactions among cat scratch patients than among normal persons. If, on the other hand, the antigenic relationship were close to that of the psitt-LGV group of agents, then one would be tempted to expect a still greater prevalence of antibodies, as well as a greater number of individuals demonstrating fourfold (or higher) antibody increases among the patients. Furthermore, few of the reporting laboratories indicate antibody increases when performing serologic tests on serums of patients with cat scratch disease. Apparently most laboratories are satisfied with the mere demonstration of antibody rather than the conventionally accepted fourfold antibody rise. It is recognized that cat scratch disease is a chronic-type illness, and often the physician does not see the patient until late in the clinical course of the disease. It would appear, however, that the number of cases with demonstrable antibody changes are fewer than should be expected. Greater numbers of individuals with antibody would also be expected as more time would have elapsed permitting development of the antibodies. In contrast, the majority of patients with positive skin tests do not manifest this CF reactivity. It is conceivable that individuals with a CF response have been in contact with an agent antigenically related to the psitt-LGV group. This agent, although related to the psitt-LGV group of viruses, may at the same time be one of several comprising the cat scratch disease etiologic spectrum. The CF reactions may not be primary reactions, but anamnestic, owing to the presence of an antigen (or antigens) common to the psitt-LGV group; or the reactions may conceivably be nonspecific.

The attempt to obtain further information on this relationship between cat scratch disease and the psitt-LGV group of viruses by skin testing LGV patients was not too rewarding. The number of patients involved was too small for clarification of the relationship. Larger numbers are obviously necessary in order to draw significant conclusions. It is recognized that cat scratch patients do not respond to the Frei antigen. The results reported here reveal that a number of LGV patients do not react to cat scratch antigen in skin tests.

TABLE V

Literature results on cat scratch disease patients tested for CF antibody to the psitt-LGV group of viruses

Investigator	Source of serums	Number tested	Number positive	Percent positive*
Manning and Reid (9)	Patients	35	8	23.0
	Pos. path.	10	6	60.0
	Neg. skin test	44	0	0.0†
	Controls	204	4	2.0
Armstrong et al. (8)	Patients	40	8	20.0
	Controls	71	8	11.3
Mollaret et al. (5)	Patients	43	20	46.5
Daniels and MacMurray (2)	Patients	12	3	25.0
Kalter et al. (3)	Patients	22	7	31.8‡
Morrissey (10)	Patients	10	7	70.0
Gifford (12)	Patients	4	1	25.0§
	Neg. skin test	5	2	40.0
Waters et al. (13)	Patients	4	2	50.0
Spaulding and Hennessy (4)	Patients	39	14	35.9
	Controls	120	0	0.0
Kalter (present study)	Patients	170	44	25.8
	Controls	200	6	3.0

*A titer of 1:10 or higher.

†One individual had a titer of 1:5.

‡Five individuals had a titer of 1:5.

§Veterinarians.

Additional difficulties arise when attempting to employ the skin-test antigen as an aid to final diagnosis. Most noteworthy are the variations in response of patients to different preparations of test antigen. Individual variation to skin-test material has become well recognized. This material is usually so limited in quantity, however, that it has been impracticable to attempt a detailed evaluation. McGovern and co-workers reported that their patients reacted positively with all antigens employed but varied as to intensity of the erythema. The data reported here, while still limited, disclose not only variation in intensity of reaction among patients, but also the complete failure of certain individuals to respond to one or another of the test preparations. The differences observed in the comparative testings suggest also that more than one strain of "virus" may be encountered. One antigen preparation (No. 4) was discarded after tests on 3 individuals were negative (the donor was not available). In retrospect, more extensive testing of patients may have yielded results similar to those observed with other lots (Nos. 7 and 11). The recent report (7) of a hemagglutination test, if confirmed, may help to differentiate the variations noted above. It would be interesting to correlate serologic responses with reactions to skin tests.

Final clarification of this problem awaits isolation of a specific agent or at least a serologic test capable of detecting antibody changes. The data described here suggest that more than one agent is responsible for this disease. If one agent is responsible, then the antigenic relationship between strains is probably quite remote. Continued study of the disease entity, especially the diagnostic considerations, is necessary. For practical purposes, it is now suggested that antigen pools be used for skin testing in order to diminish the probability of negative reactions.

CONCLUSIONS

The results reported here on the serologic responses of patients with cat scratch disease to the psitt-LGV group antigen substantiate those previously described. A group of 130 patients, positive to skin tests, demonstrated a 23.1 percent CF antibody response to the group antigen. This figure is consistently higher than those obtained (or reported) for normal control groups. In addition, variations in skin-test reactions were observed with different preparations of test antigen. These variations extended from complete failure to produce an area of erythema to marked differences in size of the wheal. Because of the extreme variation in reactivity, it is recommended that skin-testing antigen be derived from pooled material in order to decrease the probability of obtaining this variable response.

The response of a certain number of patients with production of psitt-LGV group antibodies, and the marked variation in response to different preparations of skin-test antigen observed in other patients, suggest the possibility that more than one agent is responsible for the syndrome referred to as cat scratch disease. Antigenic overlapping with the psitt-LGV group may account for the relatively high incidence of serologic reactivity among patients having cat scratch disease.

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